The cytoskeletal effector xPAK1 is expressed during both ear and lateral line development in *Xenopus*

NAZRUL ISLAM1, LUC POITRAS1 and TOM MOSS1, 2,*

1Cancer Research Centre and Department of Medical Biology, Laval University, Québec, Canada and 2LBME-CNRS UPR 9006, Toulouse cedex, France

ABSTRACT xPAK1, a probable effector of stress activated MAP-kinase SAPK1/JNK activation and cytoskeletal dynamics, was found to be ubiquitously expressed within the *Xenopus laevis* ear and lateral line system during the development and differentiation of these organs. xPAK1 expression was very strong in the otic placode from its condensation, and expression continued in the otic vesicle up until stage 35/36, after which it abruptly ceased. At stage 29/30 expression occurred specifically in the epithelium of the otic vesicle, which includes the prospective sensorial epithelium. Expression of xPAK1 was also observed in the lateral line system from stage 35/36, at which stage the lateral line primordia have begun to migrate from the region of the otic vesicle. Lateral line expression continued at least until stage 37/38, at which time xPAK1 was noted in association with the differentiating lateral line organs. To our knowledge, xPAK1 is the first ubiquitous lateral line marker that is also expressed in the ear. In the context of previous studies, our data suggest that xPAK1 either plays a role in the differentiation of the mechano-sensors of the auditory system or in the formation of the otic vesicle epithelium and the lateral line primordia.

KEY WORDS: *Xenopus, ear, lateral-line, xPAK1*

The development of the auditory system in *Xenopus* is a subject of considerable interest, presenting as it does a complex patterning of canals and chambers, the differentiation of mechano-receptors and innervation by the eighth cranial nerve (Nieuwkoop and Faber, 1967; Fritzsch, 1996). The otic placode, the ear primordium, condenses in *Xenopus* by stage 21 and develops into the closed otic vesicle by stage 27. Both auditory and vestibular mechanisms then develop quite rapidly and are probably functional by stage 45. It is not certain at what stage the hair cells, the mechano-receptors or neuromasts of the ear, begin to differentiate but at least some are fully differentiated at stage 45 (Díaz *et al.*, 1995). The lateral line organs are a complex system of epidermal mechano-receptors present in amphibia and fish. They are phylogenetically related to the hearing and vestibular apparatus, and consist of small groups of neuromast cells, each very similar to the hair cells of the ear (Nieuwkoop and Faber, 1967; Winklbauer, 1989). In *Xenopus*, each group of neuromasts develops at the base of an epidermal pit. Though molecular markers of ear development have been identified, to our knowledge, only one, Tor70, is known to be expressed both during ear development and during development of the related lateral line organs (Bolce *et al.*, 1992). Tor70 is, however, not expressed in all lateral lines. Thus, none of the known markers to date have a strong potential of encoding common factors in sensorial epithelium or neuromast differentiation.

We have found that the developmentally regulated expression of xPAK1, is associated with ear and lateral line development in *Xenopus*. The p21-activated kinases (PAKs) were one of the first targets of the activated GTPases Rac and Cdc42 to be identified (Manser *et al.*, 1994; Daniels and Bokoch, 1999; Manser and Lim, 1999). PAK is a fairly close relative of the sterile 20 (Ste20p) kinase implicated in MAP-kinase activation, cytoskeletal reorganization and cell cycle arrest, and is a more distant relative of the *Drosophila* kinase MSN implicated in planar polarity.

xPAK1 is expressed throughout early embryogenesis and in the adult

The sequence of the xPAK1 protein (xPAK1p) deduced from a cDNA identified using the RT-PCR fragment Xltk24 (Islam *et al.*, 1994) differed at only six amino acids (and the cDNA sequence differed at 26 positions) from a clone previously identified (Genbank AF000239) (Faure *et al.*, 1997) (Fig. 1A). Since both xPAK1 cDNAs showed essentially identical 5’ and 3’ flanking sequences they probably represent the same gene. Alignment with the mammalian PAKs suggested that xPAK1 was most closely related to rPAKα and hPAK1. xPAK1 structure is summarized diagrammatically in Figure 1B. xPAK1 was found to be expressed as a maternal message whose concentration reduced until well past the mid-blastula tran-
sition (Fig. 2A,B). mRNA concentration was very low at the early gastrula, stage 10 (Nieuwkoop and Faber, 1967), but picked up around neural plate stage, stage 14, and continued to increase at least as far as stages 40 to 45. Present maternally, xPAK1p protein maintained a roughly constant concentration until mid-neurula, stage 17, (Fig. 2C). At stages 21 and 25 xPAK1p was present at a low concentration, but by stage 36 its concentration had increased to well above the maternal level. In the adult, significant levels of both message and protein were present in the spleen, brain and stomach and lower levels were found in the pancreas and stomach (mRNA was not tested) (Fig. 2D,E).

Expression in the otic placode and otic vesicle epithelium

The most striking site of early xPAK1 expression was the developing ear. The otic placode is first defined after neural tube closure at stages 20/21 (Nieuwkoop and Faber, 1967), forms a closed vesicle by stage 27 and by stage 29/30 the otic vesicle is separated from the overlying epidermis. From about stage 22 (Fig. 3A) to at least as far as stage 35/36 (Fig. 4A), xPAK1 mRNA was abundant within the otic placode and later in the otic vesicle. By stage 37/38, however, expression in the ear was significantly attenuated (Fig. 4B). Sectioning of embryos at stage 29/30 showed that xPAK expression occurred only within the epithelium of the otic vesicle. This cell layer includes the sensorial epithelium, which gives rise to the hair cells of the ear. Though the exact timing of hair cell differentiation in Xenopus is not known, it probably starts around stage 29 and at least some hair cells are functional by stage 45 (Nieuwkoop and Faber, 1967).

xPAK1 has been associated with apoptosis in Xenopus oocytes (Faure et al., 1997). However, in agreement with an earlier study (Hensey and Gautier, 1998), we detected no apoptotic events associated with the development of the otic vesicle (e.g. Fig. 3I,J). Thus, it would not appear that the opening of the otic vesicle occurs by programmed cell death. The function of xPAK1p in the ear would therefore seem to be related either to the formation of the sensorial
epithelium, in part a polar epithelium of hair cells (Díaz et al., 1995), or to the differentiation of the hair cells themselves.

Expression in the extending and differentiating lateral line system

Already by stage 35/36, expression of xPAK1 was observed in the elongating lateral line system (Fig. 4A,B), temporally overlapping expression in the ear. The lateral line organs form from primordia which migrate from the region of the ear starting at about stage 33/34 (Nieuwkoop and Faber, 1967). After migration, the primordial cells differentiate into lateral line placodes which along with cells of neural crest origin become the lateral line organs (Collazo et al., 1994). Each placode [Fig. 4A-D] during development of the sensorial epithelium of this organ).

Possible functions for xPAK1

xPAK1 provides probably the first developmental marker of the ear which is also expressed ubiquitously during lateral line development. Since this expression also occurs during the process of hair cell and neuromast differentiation, it suggests a role for xPAK1 in the process of differentiation of these mechano-receptors. Both the hair cells and neuromasts develop actin-based stereocilia which rely on specialized myosins for both their development and function (Gillespie and Corey, 1997). PAK1 and xPAK1 have been shown to directly phosphorylate lower eukaryotic myosin I and vertebrate cytoplasmic myosin II in vitro, and xPAK1 has also been shown to do so in vivo (Tuazon and Traugh, 1984; Wu et al., 1996; Brzeska et al., 1997; Ramos et al., 1997, and Islam et al. submitted). Thus, a possible function for xPAK1p lies in the development of the actinomyosin based stereocilia of the neuromast cells. On the other hand, xPAK1 expression appears to be rather evenly distributed over the epithelium of the otic vesicle and it is very probable that not all this surface will generate hair cells. Further, the potential function of the PAK proteins as effectors of the Stress Activated MAP-kinase (SAPK1/JNK) pathway suggests they may fulfill a more general role in the formation of epithelia and possibly polar epithelia, (Noselli and Agnés, 1999), of which the sensorial epithelium of the ear is an example (e.g. see Díaz et al., 1995). The function of xPAK1 in the lateral lines could yet further be related to the migration of the lateral line primordia. Cell migration and the formation of polar epithelia in Drosophila, in fact, share common components and both show a dependence on SAPK1/JNK activation, (Noselli and Agnés, 1999). Thus, the role of xPAK1 may rather lay in the formation of a polar
epithelium and/or cell migration than in the subsequent differentiation of the mechanoreceptors of the ear and lateral lines.

Experimental Procedures

Cloning

A first clone of xPAK1 was isolated by the use of degenerate primers corresponding to kinase domain VIII and IX in a PCR reaction on stage 24 cDNA (Islam et al., 1994). This clone, xltk24, was then transcribed as a riboprobe and used to screen a Xenopus laevis embryonic stage 17 sg10 cDNA bank provided by D. Melton. Screening of this bank was performed in 50% formamide, 6xSSC, 5xDenhardt’s and 100 µg/mL torula RNA (Sigma) at 42°C. After hybridization, washing consisted of two washes in 6xSSC and two washes in 2xSSC at 42°C for 15 min each. The filters were then treated with RNase A, 20 µg/mL and RNase T1, 10 units/mL in 2xSSC at 37°C for 30 min before further washing in 0.5xSSC, 0.1% SDS at 55°C for 30 min. A partial cDNA was isolated and used to screen a stage 24 sg10 cDNA bank established using a degenerate primer to kinase domain IX (Islam et al., 1996). Finally, both clones were completely sequenced, fused by restriction and religation and resequenced.

Northern and RT-PCR

mRNA was isolated from staged embryos and adult tissues using guanidinium isothiocyanate and LiCl precipitation (Cathala et al., 1983). Northern blot was prepared from formaldehyde gel separation (Brown, 1996). Finally, both clones were completely sequenced, fused by restriction and religation and resequenced.

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References


